

Bulletin of the Agricultural Chemical Society of Japan.

TRANSACTIONS

Studies on Vitamin B₂ Complex. V

Further Experiments on the Effect of Carbohydrate on
Vitamin B₂ Deficiencies. Flavin Synthesis in Rats.*

By Ume TANGE.

(The Institute of Physical and Chemical Research.)

Received Nov. 13, 1939.

In the previous paper⁽¹⁾ data were submitted which showed that the type of carbohydrate employed in the basal rations was an important factor in the study of the vitamin B₂ complex. The remarkable difference in the development of the vitamin B₂ deficient symptoms in the rats fed with the diets containing sucrose, corn-starch and lactose was noticed. Especially, in the case of the lactose ration, none of the rats did develop dermatitis, although showing cataract, and they attained somewhat subnormal growth even in the entire absence of vitamin B₂ complex. These results suggested that the presence of lactose might have favoured the synthesis of vitamin B₂ complex by the bacterial flora in the rats' intestine. Moreover, the past experiments⁽²⁾ with diets containing dextrin and sucrose, deficient in vitamin B₆ but otherwise complete, showed very different effects on the onset of dermatitis; namely, that no dermatitis occurred with the dextrin ration but with a similar sucrose diet dermatitis was quite severe.

These and other observations led the author to attempt further investigations to determine whether rats can synthesize vitamin B₂ factors when the experimental diets are deficient in these factors.

EXPERIMENTAL.

The series of diets employed in the present studies was similar in composition to those previously reported,⁽¹⁾ as shown in Table I.

The methods employed in this experiment were mostly similar to those described in the previous paper,⁽¹⁾ care being taken to distribute litters and sex uniformly throughout the several groups. The young rats weighing between 45

Table I.

Composition of various rations used:

| Component \ Rations (per cent.) | Diet C | Diet S | Diet L | Diet G | Diet D |
|--|--------|--------|--------|--------|--------|
| Purified fish protein | 18 | 18 | 18 | 18 | 18 |
| McCollum's salt mixture | 4 | 4 | 4 | 4 | 4 |
| Agar-agar | 1 | 1 | 1 | 1 | 1 |
| Crisco | 9 | 9 | 9 | 9 | 9 |
| Corn-starch (commercial) | 68 | — | — | — | — |
| Sucrose (<i>Pharmacopeia Japonica</i>) | — | 68 | — | — | — |
| Lactose (" ") | — | — | 68 | — | — |
| Glucose (" ") | — | — | — | 68 | — |
| Dextrinized corn-starch† | — | — | — | — | 68 |

† Made from commercial corn-starch by moistening the starch with a 0.1 per cent. solution of citric acid, autoclaving for 5 hours at 120°C, drying and pulverizing.

to 55 g were placed in the cages provided with raised bottoms of coarse wire-screens to prevent accessibility to feces. When the weight of the animals remained stationary or declined, one drop of cod liver oil and 20 γ of vitamin B₁ hydrochloride were supplied daily.

In the case of the diets with the lactose and dextrinized cornstarch, a number of animals was placed on these diets in advance of the remaining groups in order that their feces might be available as supplements to the other groups of vitamin B₂ complex deficient diets. The growth curves of the former groups of rats were not shown in Charts, but they were similar to those of the lactose- and dextrin-diet groups, given in Charts 1 and 2.

The feces excreted by the animals receiving the lactose and dextrin rations were collected daily and stored under ether until adequate amounts were obtained. Then the feces were extracted with ether several times to remove fatty materials and pulverized. These pulverized feces were provided at level of 0.5 g daily as supplements to the vitamin B₂ complex deficient diets containing other carbohydrates than lactose. The results are shown in Charts 3 to 10.

One experiment was carried out as a continuation of the effect of lactose on the cataract-producing action. Some groups of rats were fed on a diet similar to Diet L given in Table I, but with 55% lactose and 35% fish protein or egg albumin instead of 68% lactose and 18% fish protein, supplemented likewise with vitamins B₁, A, and D. Very few of these rats showed cataract which was less complete and was greatly delayed in development. However, the growth rate of the animals was not high, but rather low, compared with that on 68% lactose and 18% protein. The addition of filtrate factor brought about obvious improvement on

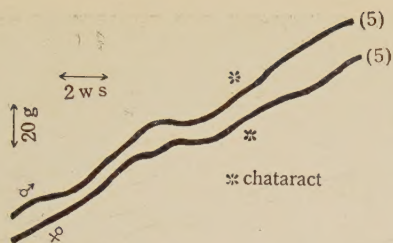


Chart 1. Average growth curves of rats on Diet L, without feces. The figures in brackets denote the number of animals considered.

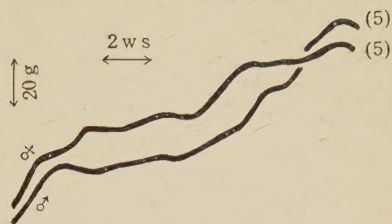


Chart 2. Average growth curves of rats on Diet D, without feces. The figures in brackets denote the number of animals considered.

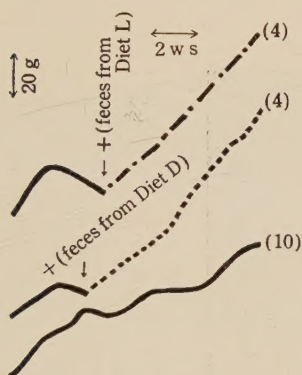


Chart 3. Average growth curves of rats on Diet D, with or without feces. The figures in brackets denote the number of animals considered.

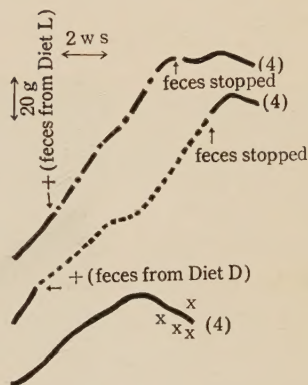


Chart 4. Average growth curves of rats on Diet C, with or without feces. The figures in brackets denote the number of animals considered, x died.

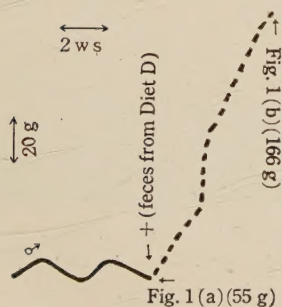


Chart 5. Growth curve of rats on Diet C, supplemented with feces from Diet D rats. (Refer to Figs. 1, (a) and (b).)

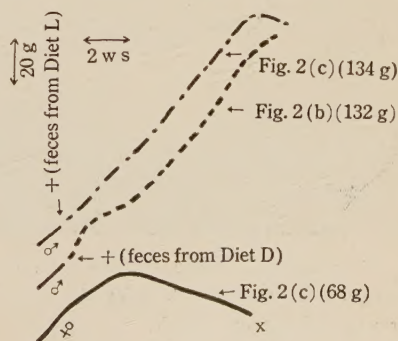


Chart 6. Growth curves of rats on Diet C, with or without feces, x died. (Refer to Figs. 2, (a), (b) and (c).)



Fig. 1. (a) Showing the subnormal condition of the rat on Diet C, not supplemented with feces (body wt. 55 g).



(b) Showing the normal health and growth after about 5 weeks of administration of the feces from Diet D rats on the same rat (a) (body wt. 166 g).



Fig. 2. (a) Showing the subnormal condition of the rat on Diet C, not supplemented with feces.



(b) Showing the influence of feces from Diet D rats on the rat fed with Diet C, to promote growth and improve health.



(c) Showing the influence of feces from Diet L rats on the rat fed with Diet C, to induce normal health and growth.

These rats are litter mates. They were photographed on the fifty-fourth day of the experiment, at which time they weighed 68 g (a), 132 g (b), and 134 g (c), respectively.

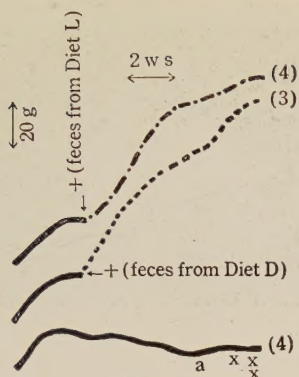


Chart 7. Average growth curves of rats on Diet G, with or without feces. a acrodynia, x died. The figures in brackets denote the number of rats considered.

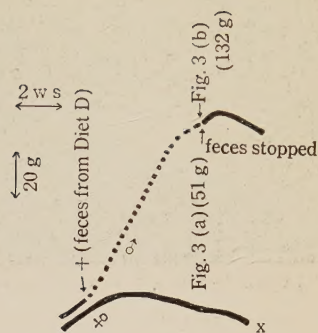


Chart 8. Growth curves of rats on Diet G, with or without feces.
(Refer to Figs. 3, (a) and (b).)



Fig. 3. (a) Showing the unhealthy appearance of the rat on Diet G, not supplemented with feces.



(b) Showing the favourable growth effect of the feces from Diet D rats on the rat fed with Diet G.

These rats are litter mates. They were photographed on the forty-second day, at which time they weighed 51 g (a) and 132 g (b), respectively.

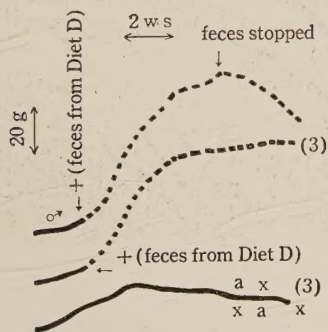


Chart 9. Average growth curves of rats on Diet S, with or without feces. a acrodynia, x died. The figures in brackets denote the number of rats considered.

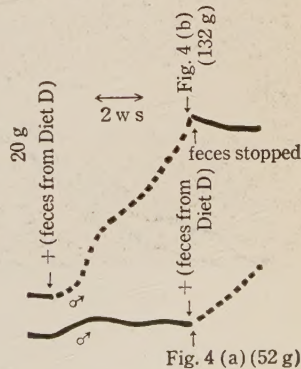


Chart 10. Growth curves of rats on Diet S, with or without feces.
(Refer to Figs. 4, (a) and (b).)



Fig. 4. (a) Showing the unhealthy appearance of the rat on Diet S, not supplemented with feces.



(b) Showing the favourable growth effect of the feces from Diet D rats on the rat fed with Diet S.

These rats are litter mates. They were photographed on the forty-ninth day, at which time they weighed 52 g (a) and 132 g (b), respectively.

the growth. In this case, the inhibitory action on cataract was attributed to the effect of the amount of protein rather than that of lactose, since 55% lactose and 18% protein in a similar diet had nearly the same degree of cataract production (unpublished) as in 68% lactose and 18% protein.

Fully developed cataract occurred in nearly 100% with the ration containing 68% lactose and 18% fish protein and the average time required for its production was 10 weeks, while with the ration containing 55% lactose and 35% fish protein or egg albumin, the cataractous change in the lens appeared in only insignificant degree in the experimental period of about 18 weeks, except in two out of sixteen rats which showed marked cataract. These relations are shown in Chart 11.

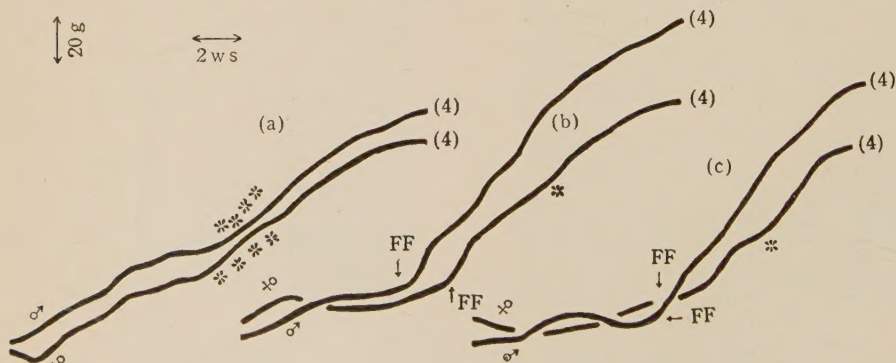


Chart 11. Average growth curves of rats on diets containing 18% fish protein (curve a), 35% egg albumin (curve b), and 35% fish protein (curve c), respectively.

FF filtrate factor, * mature cataract. The figures in brackets denote the number of animals considered.

RESULTS AND DISCUSSION.

From the above results, it appeared evident that the groups of rats which received feces, excreted by the animals fed with lactose- and dextrin-diet showed a much more favourable growth response than comparable groups of animals which received the respective diets unsupplemented. This beneficial effect of lactose and dextrinized corn-starch was considered to be due to the action of the intestinal flora of the rats. With the examination of the feces this condition was found to be true. We observed a remarkable contrast in the nature of the feces excreted by the animals receiving the lactose and dextrin diets, compared to the feces voided by the animals receiving sucrose-, glucose-, or corn-starch-diet. The feces from the animals fed on the former two diets (lactose and dextrin) were usually large, moistened, bulky pellets, while those from the animals fed on the latter three diets (sucrose, glucose, and corn-starch) were hard, small, black pellets of commonly irregular form. It seemed, therefore, to be possible that the different nature of these two sets of groups mentioned above, was attributable to the results of the action of certain microorganisms that inhabited the digestive tract of the animals. On autopsy examination of such animals, the cecum of the lactose- and dextrin-fed was unvariably found to be distended and filled with residual dietary materials, in contrast to the contracted and empty cecum of the animals which received the sucrose, glucose, or corn-starch as the source of carbohydrate.

As the results of the above finding, an attempt to isolate flavin from the feces was made with the hope of ascertaining the components of vitamin B₂ implicated. The determination of flavin in the feces was made by the methods of Kuhn.⁽³⁾ The ether extracted residue (about 60 g as dry powder) of feces mentioned above, was extracted three times with 80% methanol. The combined methanol solution was concentrated under reduced pressure, and this concentrated solution was extracted first with ether, and then with chloroform to remove fatty materials and pigments. After the aqueous solution was separated from ether and chloroform, it was acidified with HCl to pH 3.0, and then adsorbed twice on acid clay. The united adsorbates were eluted by shaking with a mixture of pyridine-methanol-water (1:1:3). After similar treatment was repeated twice more, the eluates were combined and evaporated in vacuo until pyridine was completely removed. These procedures were all carried out protected from light. The evaporated residue was diluted with H₂O and made to 0.5 N alkaline solution with NaOH. This alkaline solution was irradiated by passing air current on 500 W electric lamp at a distance of 20 cm below 20°C for 2 hours. The resulted lumiflavin was acidified with HCl, and extracted several times with CHCl₃. The combined chloroform extracts were dehydrated with anhydrous sodiumsulphate and concentrated to a definite volume under diminished pressure, then lumiflavin was estimated by Zeiss' Pulfrich Photometer. The amounts of flavin calculated from lumiflavin were about 300 micrograms. Their absorption spectra are given below.

Such findings as above led the author to determine whether the respective

rations used in these experiments carried appreciable amounts of flavin and whether the presence of untreated corn-starch in the rats' intestine favoured also the bacterial flora to synthesize flavin. It was found, however, that none of them contained flavin. This indicated clearly that the diets containing lactose and dextrinized corn-starch affected the intestinal activities very differently from those containing sucrose, glucose, and corn-starch as source of carbohydrate. It was highly interesting to find that the dextrinized corn-starch had a favourable influence on bacterial flora in the rats' intestine, and that the untreated corn starch failed to show any such properties.

Thus data showed quite conclusively that the beneficial effects of lactose and dextrinized corn-starch on vitamin B₂ deficiencies were to be attributed to the nature of these two carbohydrates to favour the production of these factors by microorganisms in rats' intestine. Flavin was isolated from the feces from the animals fed with the two diets. It was not correct to judge, however, that flavin was the only factor contained in the feces, since sufficient amounts of vitamin B₂ factors were supplied by the feces to produce satisfactory growth comparable to that obtained by providing flavin, B₆ and "filtrate" factor to similar vitamin B₂ deficient diets as shown in the previous experiments.⁽¹⁾ When the feeding of feces was stopped, the animals declined in weight, with poor appearance of the fur and skin. Guerrant⁽⁴⁾ et al. showed that live yeast cells existed in the cecum of dextrin-fed rats in abundance, and those microorganisms were the specific agents to produce the B vitamins. Moreover, Bechdel and his co-workers⁽⁵⁾ isolated bacteria from the dried matter of cows' rumen and designated them *Flavobacterium Vitarumen*, and the microorganisms had very high ability to synthesize the vitamin B complex.

Absorption spectra.— The absorption spectra of the lumiflavin obtained from the feces, voided by the animals fed on lactose and dextrinized corn-starch diets, were very similar to those of lactoflavin estimated by Kuhn,⁽⁶⁾ even though the maximum points were not distinct like the pure lactoflavin, which might be due to contamination by some impurities. They are given below for the comparison :

| | | |
|------------------------------|------------------------------|------------------------------|
| $\lambda = 4450 \text{ \AA}$ | $\lambda = 4450 \text{ \AA}$ | $\lambda = 4450 \text{ \AA}$ |
| $\lambda = 3650 \text{ \AA}$ | $\lambda = 3800 \text{ \AA}$ | $\lambda = 3770 \text{ \AA}$ |
| $\lambda = 2650 \text{ \AA}$ | $\lambda = 2900 \text{ \AA}$ | $\lambda = 2700 \text{ \AA}$ |
| $\lambda = 2200 \text{ \AA}$ | $\lambda = 2600 \text{ \AA}$ | $\lambda = 2500 \text{ \AA}$ |

H₂O solution of
lactoflavin.

By Kuhn.

CHCl₃ solution of
lumiflavin from feces
of lactose diet (Fig.
A).

By the author.

CHCl₃ solution of
lumiflavin from feces
of dextrinized corn-
starch diet (Fig. B).

By the author.

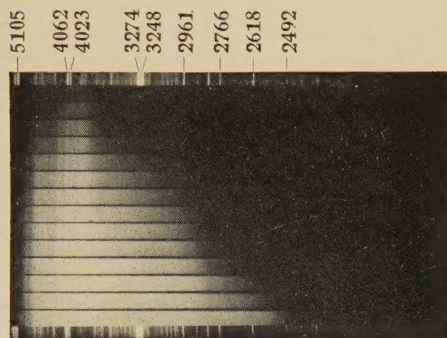


Fig. A. $1/30000$ M. CHCl_3 solution of lumiflavin isolated from the feces on Diet L rats.

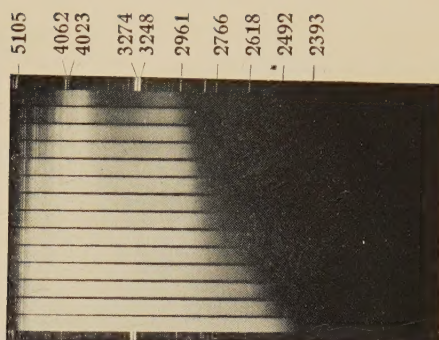


Fig. B. $1/30000$ M. CHCl_3 solution of lumiflavin isolated from the feces on Diet D rats.

SUMMARY.

1. Data are presented which show that in the vitamin B_2 deficiency the groups of rats receiving feces, voided by the animals fed on lactose and dextrinized corn-starch diets, showed a much more favourable growth response than comparable groups of animals receiving sucrose, glucose, and corn-starch.

2. The peculiar properties of the lactose and dextrinized starch are attributed to the formation of vitamin B_2 factors by microorganisms in the intestine of the rats.

3. Flavin is isolated from the feces of such rats.

4. Evidence indicates that the increased level (35%) of either fish protein or egg albumin more greatly inhibits the cataractous change in the lens than 18% level of protein.

I wish to thank Prof. U. Suzuki and Prof. B. Suzuki for their advice and encouragement during the progress of this work, and to Dr. M. Sumi for his helpful suggestions. I am also very grateful to Dr. S. Kato for the spectroscopic assay, to Miss T. Akaho for the lumiflavin determinations, and to Dr. Y. Akutagawa, of Medical Department of Jikei University, for the ophthalmoscopic study on the lens change of the animals. I am indebted to Misses M. Takahashi and H. Sasaki for their willing help in feeding the animals and preparing the materials.

* This paper was presented at the Scientific Meeting of I. P. C. R., June 16, 1939.

- (1) U. Tange: Sc. Pap. I. P. C. R., **35**, 64 (1939).
- (2) U. Tange: Rikwagaku-kenkyu-jo Iho, **16**, 1058 (1937).
- (3) R. Kuhn, T. Wagner-Jauregg and H. Koltschmitt: Ber., **67**, 1452 (1934).
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- (5) S. I. Bechdel, H. E. Honeywell, R. A. Dutcher and M. H. Knutsen: J. Biol. Chem., **80**, 231 (1928).
- (6) R. Kuhn, P. György and T. Wagner-Jauregg: Ber. Chem. Ges., **66**, 1035 (1933).

ABSTRACTS

from

TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noticed)

**On a New Polypeptide Isolated from *Eisenia Bicyclis*.
(Part II)**

A Study of the Chemical Structure of Eisenin. (1)

(pp. 1~6)

By Tosihiro OOHIRA.

(Agricultural Chemical Laboratory, Tokyo Imperial University ;

Received Dec. 5, 1939.)

It has been described in the previous paper that "Eisenin", a tripeptide isolated from *Eisenia Bicyclis*, has the composition $C_{13}H_{20}O_6N_4$, and yields glutamic acid (2 mols), alanine (1 mol) and ammonia (1 mol) as the ultimate hydrolysis products. It has also been confirmed that eisenin contains each one of free carboxyl- and acidamide-group.

In the present paper, some noteworthy data for determining the chemical structure of eisenin are reported.

When eisenin was heated with 3% aqueous barium hydroxide solution on a boiling water-bath, its partial hydrolysis took place, evolving almost quantitatively one equivalent of ammonia and leaving a syrupy substance which gave ninhydrin reaction contrary to the original substance and still intensive biuret reaction.

By estimating acidity and amino nitrogen content of the syrupy substance obtained above, it was shown that one amino group and 2 more carboxyl groups (3 free carboxyl groups in total) per molecule of eisenin became free.

In order to confirm whether this partial hydrolysis product consists mainly of tripeptide or mixture of amino acids, or amino acid and dipeptide, and also to investigate the arrangement of the amino acids in the peptide, it was submitted to the oxidation by means of nitrous acid as well as hydrogen peroxide according to the methods used by Kendall, McKengie and Mason or by Quastel and Stewart for the study of glutathione.

On oxidation with nitrous acid, neither α -oxy-glutaric acid nor lactic acid was detected directly in the reaction product, indicating that there was no contamination with amino acid; after a complete hydrolysis, however, *dl*- α -oxy-glutaric acid, *dl*-glutamic acid and *dl*-alanine were isolated from the oxidised solution.

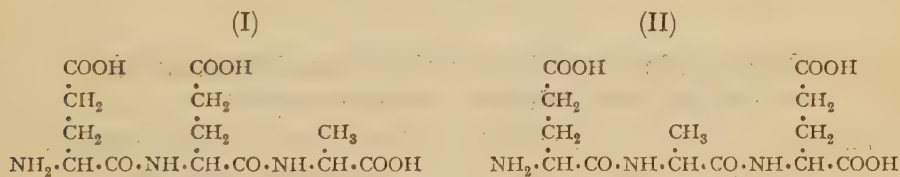
Therefore it is thought that one of the two glutamic acid molecules is attached through its carboxyl group and not through its amino group, also that the amino groups of another glutamic acid and alanine are substituted.

In the case of the reaction towards hydrogen peroxide, succinic acid and acetic acid were isolated by extracting the solution with ether directly after oxidation. From the solution exhausted with ether no more of these organic acids could be obtained though the solution was hydrolised with sulphuric acid.

This result shows that the glutamic acid, in this tripeptide molecule, is always attached to the amino group of the other amino acid by the carboxyl group which is next to the amino group.

But it is not possible to determine, by these reactions, whether only amino group or both amino and carboxyl groups in the alanine are concerned in the peptide connection.

From these results, it is concluded that the reaction product yielded by partial hydrolysis of eisenin may be a tripeptide which is denoted by either *di*-[α -amino- γ -carboxy-butyryl]-alanine (I) or [α -amino- γ -carboxy-butyryl]-alanyl-glutamic acid (II).



Work is now in progress on the structure of eisenin, the results of which will be shortly communicated.

Studies on the Yeasts Found in "Miso."

(Supp. Contribution)

Part 4. Discussion and Conclusion.

(pp. 7~17)

By Masatoshi MOGI.

(The Brewing Laboratory of Noda Shoyu Co. Ltd., Noda-machi, Chiba-ken, Japan;

Received Dec. 11, 1939.)

The author has isolated 13 new strains of yeast from 20 samples of "Miso" produced by this company and in various districts of this country.

Morphological and physiological properties of the yeasts were investigated in detail and they were accordingly classified as follows:—

Saccharomyces miso δ nov. sp.

" " var. 1 nov. sp., nov. var.

" " var. 2 " "

" *miso* ϵ nov. sp.

Zygosaccharomyces miso γ nov. sp.

" " var. 1 nov. sp., nov. var.

" " var. 2 " "

Pseudohansenula miso nov. genus, nov. sp.

Pichia miso nov. sp.

Torulopsis miso δ nov. sp.

" *miso* ϵ " "

" *uvae* (Pollacei et Nannizzi) Lodder var. *miso* nov. var.

Pseudomycoderma miso nov. sp.

The author wishes to express his deep gratitude to Prof. emeritus Dr. T. Takahashi and Prof. Dr. K. Sakaguchi for their kind advice and encouragement throughout this work.

Biochemical Studies on the Sexual Organs of the Silk Worm, *Bombyx mori* L.

Part IV. On the quantitative development and the catalase actions
of the slimy gland, appendages of the female sexual organ.

(pp. 18~22)

By Takeo NAKASONE.

(Mie Prefectural Sericultural Experiment Station; Received Dec. 1, 1939.)

Studies on the Preparation of Unsaturated Higher Fatty Alcohol by the High Pressure Hydrogenation in the Presence of Zinc Catalyst.

Part III. On the Reduction of the Methylsters of the
Mixed Fatty Acids from Soya Bean Oil.

(pp. 23~26)

By Yuichi SHINOZAKI and Shizuo SUMI.

(Dept. of Organic Chemistry, The Central Laboratory, South Manchuria Railway Co.,
Dairen; Received Nov. 21, 1939.)

Nutritive Value of Cereals and Tubers.

(Studies on Rural Foods. I.)

(pp. 27~35)

Hisayoshi IWATA.

(Morioka Imperial College of Agriculture and Forestry, Japan;

Received Dec. 16, 1939.)

Various kinds of polished cereal grains, dried tubers and chestnuts powder were compared as to their food values by using them as basal diets in feeding experiments on young albino rats.

On the Reaction and Line Status of Apple Orchard Soil in South-Manchuria.

(pp. 36~38)

By R. KAWASHIMA.

(Agr. Chem. Laboratory, Kyushu Imp. University; Received Dec. 20, 1939.)

The author has determined both the reaction and degree of lime saturation of several apple orchard soils in Ryoto peninsula of South-Manchuria. The pH values of many soils now examined are slightly over 7 and the degrees of lime saturation are generally more than 80.

On the other hand, the apple orchard soils of Nagano and Aomori in Japan are acid in reaction almost unexceptionally, and lime saturation is low. As the varieties of apple cultivated are the same between Japan and Ryoto peninsula, a question arises which of these two opposing characters of soil conditions is most suitable for apples.

In the author's opinion, it is necessary to apply more lime for the apple orchard in Japan and make the soil less acid.

On the Retting of Vegetable Fibre Materials.

Part XI. The Useful Anaerobes for the Bacterial Retting of Flax.

(pp. 39~42)

By Tosio NAKAHAMA.

(Kanebo Yamashina Institute; Received Nov. 29, 1939.)

Nearly the same effective retting of flax was attained by the anaerobic process as was previously observed with the aerobic bacteria (see Part IX and X), and eleven strains of anaerobic bacteria were isolated from the retting vat.

After carrying out pure fermentation of flax with each of these eleven strains of bacteria, one strain of bacillus and one strain of coccus were selected as the most useful organisms.

One of the useful anaerobes was classified as a new species and named *Micrococcus linumus*, since the characteristics of the bacteria were found not to be the same as those of *Micrococcus minimus* Giselli, in the propagation on milk or potato and for the sources of nitrogen.

The other useful anaerobe was found to reveal similar characteristics to *Bacillus aurantius* Sack. However, cellulose was never decomposed and the saccharification of starch was not remarkable by the bacteria. For the fermentation products, acetone or butyric acid was not detected.

It was therefore concluded that this bacteria was also a new species and it was named *Bacillus linumus*.

On the Hydrolysis of Fats and Fatty Acid Esters. (V)

(pp. 43~54)

By Toyoki ONO.

(Chemical Laboratory of the Fish Meal Association of Japan;

Received Dec. 21, 1939.)

I. Preparation of Triglycerides.

(A). Caprylic, capric, lauric, myristic, arachidic, erucic, ricinoleic, linolic, linolenic, $C_{11}H_{21-6}O_2$, and clupanodonic acid were isolated from cocoanut oil, peanut oil, rape oil, castor oil, linseed oil and sardine oil. Purified palmitic, stearic and oleic acid from commercial products.

(B). Simple triglycerides were obtained by Berthelot's method with these fatty acids—on heating for 5 hours at $120\sim 200^\circ C$ the mixture of glycerol, an excess of fatty acids and a small quantity of Twitchell's reagent.

II. Hydrolysis of Triglycerides by Pancreas Lipase.

(A). On the triglycerides of saturated fatty acids ($C_8\sim C_{18}$), the reaction velocity on hydrolysis diminishes in proportion to the molecular weight.

(B). On the triglycerides of unsaturated fatty acids (C_{18} and C_{22}), however, there is no relation between the reaction velocity and the molecular weight, but at lower temperature the reaction velocity depends upon the number of double bond (unsaturation) in glyceride.

III. Comparison of Hydrolysis between Oils and Glycerides.

Previous work showed that the saturated fats such as cocoanut oil, butter fat and beef tallow are much less hydrolysed at lower temperature than the unsaturated

ones such as perilla oil, whale oil and sardine oil.

From these experimental results it will be understood that the difference of reaction velocity, especially at lower temperature, depends upon the chemical composition of fat and oil—the contents of saturated or unsaturated glycerides. Table V. shows distinctly this explanation.

Table V. The Temperature Coefficient on Hydrolysis of Fats and Triglycerides.

| Fat or Oil | k/k' | Fat or Oil | k/k' | Triglyceride | k/k'' | Triglyceride | k/k'' |
|--------------|--------|-----------------|--------|--------------|---------|--------------|---------|
| Linseed oil | 7.45 | Castor oil | 3.46 | Caprylin | 8.14 | Olein | 8.14 |
| Perilla oil | 5.22 | Chicken fat | 7.81 | Caprin | 12.44 | Erucin | 9.80 |
| Olive oil | 6.93 | Whale oil | 3.51 | Laurin | 17.15 | Linolin | 7.02 |
| Beef tallow | 9.10 | Sardine oil | 5.96 | Myristin | 17.37 | Linolenin | 7.72 |
| Butter fat | 9.14 | Shark liver oil | 4.27 | Palmitin | 15.31 | Clupanodonin | 5.03 |
| Cocoonut oil | 11.73 | Cod liver oil | 5.93 | Stearin | 9.36 | Ricinolein | 4.46 |

k, k', k'' represent the reaction velocity coefficient at $30^{\circ}, 0^{\circ}, -10^{\circ}\text{C}.$

The Influence of Monochromatic Lights on the Action of Enzymes.

[Report XXX~XXXIII]

Especially on the Influence of Infra-red Rays.

(pp. 55~63)

By Reitaro MURAKAMI.

(Agricultural College, Utunomiya; Received Dec. 22, 1939.)

In order to further investigate the influence of infra-red rays on the enzymes in yeast, the enzyme solutions containing saccharase, amylase, proteinase and lipase respectively were irradiated by infra-red rays from a "Vim Ray" red lamp. The treatments after the addition of the enzyme solutions into the substrates were the same as described in the author's previous papers.⁽¹⁾

In this experiment, the action of the yeast saccharase was found to be promoted by infra-red rays. The saccharase was more promoted by the rays containing both infra-red and visible.

The amylase, proteinase and lipase were influenced very slightly by the action of lights. However, the enzymes were promoted by infra-red rays and the rays containing both infra-red and visible.

(1) Bull. Agri. Chem. Soc. (Japan), 176, 435~444 (1939).

Phosphoric Acid Absorbtion of Soils in Tyosen. V.

(pp. 64~70)

By MISU-Hideo.

(Agricultural Experiment Station, Government General of Tyosen ;

Received Aug. 28, 1939.)

Beiträge zur Kenntnis der Chemie des Muskeleiweißes.

I. Mitteilung. Über die Stickstoffverteilung des Kaninchenmuskelplasmas.

(ss. 71~81)

Von M. KADATSU.

(Aus dem Agrikulturchemischen Institut der Kaiserlichen Universität

Tokyo. Vorstand: Prof. Dr. E. Hiratsuka.)

(Eingegangen am 26, Dez. 1939.)

Zusammenfassung.

Mit etwa 2 kg. schweren männlichen Kaninchen wurden die folgenden Untersuchungen angestellt.

1) Nach einigen Versuchen mit der Muskulatur der hinteren Extremitäten wurde eine Methode angewandt, in der das Muskelplasma mittels Zentrifugalmaschine ungefähr quantitativ getrennt und bestimmt werden konnte. Dabei bestimmte der Verfasser das Prozent des getrennten Muskelplasmagewichtes zur ursprünglichen Muskulatur des Grades der Muskelplasmatrennbarkeit und deutete es als ein Zeichen von Muskelfleischzustandsänderung.

2) An fünf Lokalitäten der hinteren Extremitäten, an drei vom Rückgratsmuskel und an zwei von den Vordergliedern der drei oben erwähnten Kaninchen, die blutig durch Gnickschlag getötet wurden, ließ sich nach einer Probeentnahme nach einer Lagerung von 18, 48 beziehungsweise 118 Stunden im Eisschrank (0~4°C) der Muskelplasmatrennbarkeitsgrad und der Gesamt-, Rest-, Amino- (nach Folin), Ammoniakstickstoffgehalt (nach Parnas-Heller) im Muskelplasma des Muskelfleisches derselben bestimmen.

3) Der Muskelplasmatrennbarkeitsgrad ändert sich nicht nur nach den Individuen, sondern auch der Muskellokalität und hat die Neigung, an beiden Muskelenden geringer als am Mittelteil zu sein. Seine Werte vergrößern sich in der Reihenfolge: Rückgrats-, hintere Extremitäten- und Vordergliedmuskeln; sie sind besonders groß an den hinteren Enden der ersteren.

4) Der Gesamt- und Eiweiß- (koagulierbare) Stickstoffgehalt des Muskelplasmas ist im allgemeinen geringer an beiden Enden des Muskels als am Mittelteil, diese Neigung äußert sich klar an den Rückgratsmuskeln, aber nicht an

den hinteren Extremitätenmuskeln. Zu dieser Tatsache wird der Zusammenhang von Muskelform und Tätigkeit erörtert.

5) Rest-, Amino- und Ammoniak-stickstoffgehalt zeigen dieselbe Neigung an den hinteren Extremitäten wie der Gesamtstickstoffgehalt; an den hinteren Enden des Rückgratsmuskels ist das Verhältnis jedoch ein umgekehrtes.

6) In der Stickstoffverteilung im Muskelplasma beträgt der Eiweißstickstoff an den hinteren Extremitäten 79~83% (Reststickstoff 17~21%) und variiert mehr und mehr vom Mittelteil nach den beiden Enden. Es ist jedoch beachtenswert, daß der Reststickstoff an den hinteren Enden des Rückgratsmuskels 35~42% des Gesamtstickstoffes erreicht.

Amino- (5~7%), Ammoniak- (1~3%) stickstoffverteilung sind an den Lokalisationen groß, an denen der Reststickstoff groß ist.

7) Die Variierung der oben erwähnten Werte zwischen den Muskellokalitäten des hinteren Extremitätenmuskels ist am nächsten zu den experimentellen Fehlergrenzen, wenn dieselben auf beide Enden oder auf das hintere Ende entfallen, und an diesem Punkt läßt sich die Homogenität der hinteren Extremitätenmuskeln erkennen.

On the Formation of Ascorbic Acid from Mannose in Plants and in Animal Bodies. IV.

(pp. 82~83)

By Tetutaro TADOKORO and Tuneyuki SAITO.

(Hokkaido Imperial University; Received Dec. 16, 1939.)

On the Formation of Ascorbic Acid from Mannose in Plants and in Animal Bodies. V.

(pp. 84)

By Tetutaro TADOKORO.

(Hokkaido Imperial University; Received Dec. 16, 1939.)

The Effect of Glutathione upon Narcotism.

(Biochemical Studies on Glutathione. The IXth Report.)

(pp. 85~102)

By Masayoshi OGAWA.

(Department of Nutrition, College of Medicine, Nippon University;
Received Nov. 24, 1939.)

In this report the author described an experiment on the effect of glutathione

upon narcotism, employing a number of male albino rats weighing from 100 grams to 150 grams, which were narcotised by subcutaneous injections of bromral (30 mg per 100 grams of the body weight) and obtained the following results.

Rate of Sleep.

| The time of the inj. of GSH after the inj. of bromral. | GSH (mg) injected (per 100 grams of the body weight) | | | | | | | | |
|---|--|-----------|-----------|-----------|-----------|-----------|------------|------------|------------|
| | 0 mg | 0.1 mg | 0.5 mg | 1.0 mg | 2.5 mg | 5.0 mg | 10.0 mg | 25.0 mg | 50.0 mg |
| 1 hr before the inj. of bromral. | 100 | 68 | 68 | 135 | 129 | 135 | 178 | 166 | 201 |
| At the same time. | 100 | 123 | 148 | 203 | 197 | 221 | 185 | 166 | 209 |
| Injected 1 hr after the inj. of bromral. | 100 | 123 | 197 | 203 | 172 | 166 | 215 | 184 | 191 |
| Inj. 2 hrs after the injection of bromral. | 100 | 116 | 178 | 240 | 227 | 209 | 233 | 178 | 203 |

As shown in the above table, the animals injected with bromral and GSH slept soundly and more deeply than the animals which were injected with bromral only.

By injecting a large dose such as 100 mg. of bromral per 100 grams of the body weight, and at the same time, injecting GSH in doses of 0 mg., 5 mg., 10 mg., 20 mg. and 30 mg. respectively per 100 grams of the body weight, the author obtained the following results:

Death or Recovery of the Animals.

| Body weight (gram) | Bromral (mg) injected | GSH (mg) injected | Doses of GSH (per 100 grams of the body weight) | Death | Recovery |
|-----------------------|--------------------------|----------------------|---|-------|----------|
| 102 | 90 | 0 | 0 | + | - |
| 149 | 149 | 0 | 0 | + | - |
| 130 | 130 | 0 | 0 | + | - |
| 112 | 110 | 0 | 0 | + | - |
| 174 | 175 | 9 | 5 | + | - |
| 145 | 150 | 15 | 10 | + | - |
| 146 | 150 | 15 | 10 | + | - |
| 136 | 140 | 14 | 10 | + | - |
| 170 | 170 | 17 | 10 | + | - |
| 152 | 150 | 23 | 15 | - | + |
| 172 | 170 | 25 | 15 | - | + |
| 124 | 120 | 25 | 20 | - | + |
| 155 | 150 | 30 | 20 | - | + |
| 147 | 150 | 30 | 20 | - | + |

| | | | | | |
|-----|-----|----|----|---|---|
| 164 | 160 | 33 | 20 | + | - |
| 149 | 150 | 50 | 30 | - | + |
| 149 | 150 | 45 | 30 | - | + |
| 169 | 170 | 50 | 30 | - | + |

As shown in the above table, all the animals injected with 0 mg., 5 mg., or 10 mg. of GSH per 100 grams of the body weight died (death rate was 100%), but only 11% of the animals injected with 15 mg., 20 mg., or 30 mg. of GSH per 100 grams of the body weight died.

A Study on Bacteria of Korean Soy Preserved-Crabs.

(pp. 103~126)

By Y. L. Pak M. D.

(Seoul, Chosen (Korea); Received Oct. 28, 1939.)

This is report on the study of bacteria isolated from the various parts of the soy-preserved crab, the liver, generative organs, leg muscles. This long preserved crab is a favorite dish for Koreans.

A study was made on the bio-chemical nature, mode of growth, fermentative actions and also on the fermentation products of the bacteria isolated, the identities and varieties of which were as follows:—

1. *B. megatherium* var. K. S. C.
2. *B. mycoides* var. K. S. C.
3. *B. mesentericus* var. K. S. C. No. 1.
4. *B. mesentericus* var. K. S. C. No. 2.
5. *B. fusiformis* var. K. S. C. No. 1.
6. *B. fusiformis* var. K. S. C. No. 2.
7. *B. panis* var. K. S. C.
8. *B. lentus* var. K. S. C. No. 1.
9. *B. lentus* var. K. S. C. No. 2.
10. *B. spinosporus* var. K. S. C. No. 1.
11. *B. spinosporus* var. K. S. C. No. 2.
12. *B. agri* var. K. S. C.
13. *B. teres* var. K. S. C.
14. *B. simplex* var. K. S. C. No. 1.
15. *B. simplex* var. K. S. C. No. 2.
16. *Phytomonas fluccumfaciens* var, K. S. C. No. 1,

17. *Phytomonas fluccumfaciens* var. K. S. C. No. 2.
 + 18. *Mic. epimetheus* var. K. S. C.
 + 19. *Mic. aurantiacus* var. K. S. C.

As shown in the above table, all the animals injected with 0.5 mg. of 10 mg. of GST per 100 grams of the body weight died (death rate was 100%), but only 11% of the animals injected with 15 mg., 20 mg. or 30 mg. of GST per 100 grams of the body weight died.

A Study on Bacteria of Korean Soy Preserved-Crabs

(pp. 103-106)

By Y. L. Park, M.D.

(Dept. of Microbiology, Seoul National University, Seoul, Korea)

This is a report on the study of bacteria isolated from the various parts of the soy-preserved crab, the liver, reproductive organs, leg muscles. This food preserved crab is a favorite dish for Koreans.

A study was made on the bacteriological nature of growth characteristics and on the fermentation products of the bacteria isolated. The identities and varieties of which were as follows:

1. *B. megaterium* var. K. S. C.
2. *B. mycoides* var. K. S. C.
3. *B. mesentericus* var. K. S. C. No. 1
4. *B. mesentericus* var. K. S. C. No. 2
5. *B. fusiformis* var. K. S. C. No. 1
6. *B. fusiformis* var. K. S. C. No. 2
7. *B. pasteurii* var. K. S. C.
8. *B. lactis* var. K. S. C. No. 1
9. *B. lactis* var. K. S. C. No. 2
10. *B. spinospora* var. K. S. C. No. 1
11. *B. spinospora* var. K. S. C. No. 2
12. *B. agri* var. K. S. C.
13. *B. laevis* var. K. S. C.
14. *B. stibiles* var. K. S. C. No. 1
15. *B. stibiles* var. K. S. C. No. 2
16. *Phytomonas fluccumfaciens* var. K. S. C. No. 1